

Technologies, Inc.) in Opti-MEM, serially diluted to the desired concentrations, and applied to washed cells. Basal and untreated (no oligonucleotide) control cells were also treated with Lipofectin. Cells were incubated for 4 h at 37 °C, at which time the medium was removed and replaced with standard growth medium with or without 5 mg/mL TNF- $\alpha$  7 & D Systems). Incubation at 37 °C was continued until the indicated times.

***Quantitation of ICAM-1 Protein Expression by Fluorescence-activated Cell Sorter***

[0273] Cells were removed from plate surfaces by brief trypsinization with 0.25% trypsin in PBS. Trypsin activity was quenched with a solution of 2% bovine serum albumin and 0.2% sodium azide in PBS (+Mg/Ca). Cells were pelleted by centrifugation (1000 rpm, Beckman GPR centrifuge), resuspended in PBS, and stained with 3  $\times$  10<sup>5</sup> cells of the ICAM-1 specific antibody, CD54-PE (Pharmingen). Antibodies were incubated with the cells for 30 min at 4°C in the dark, under gently agitation. Cells were washed by centrifugation procedures and then resuspended in 0.3 mL of FACSFlow buffer (Becton Dickinson) with 0.5% formaldehyde (Polysciences). Expression of cell surface ICAM-1 was then determined by flow cytometry using a Becton Dickinson FACSscan. Percentage of the control ICAM-1 expression was calculated as follows: [(oligonucleotide-treated ICAM-1 value) - (basal ICAM-1 value)]/(non-treated ICAM-1 value) - (basal ICAM-1 value)]. (Baker, Brenda, *et. al.* 2'-O-(2-Methoxy)ethyl-modified Anti-intercellular Adhesion Molecule 1 (ICAM-1) Oligonucleotides Selectively Increase the ICAM-1 mRNA Level and Inhibit Formation of the ICAM-1 Translation Initiation Complex in Human Umbilical Vein Endothelial Cells, *The Journal of Biological Chemistry*, 272, 11994-12000, 1997.)

[0274] ICAM-1 expression of chimeric C3'-endo and C2'-endo modified oligonucleotides of the invention is measured by the reduction of ICAM-1 levels in treated HUVEC cells. The oligonucleotides are believed to work by RNase H cleavage mechanism. Appropriate scrambled control oligonucleotides are used as controls. They have the same base composition as the test sequence.

[0275] Sequences that contain the chimeric C3'-endo (2'-MOE) and C2'-endo (one of the following modifications: 2'-S-Me, 2'-Me, 2'-ara-F, 2'-ara-OH, 2'-ara-O-Me) as listed in Table X below are prepared and tested in the above assay. SEQ ID NO: 24, a C-raf targeted oligonucleotide, is used as a control.

Table X

## Oligonucleotides Containing chimeric

## 2'-O-(2-methoxyethyl) and 2'-S-(methyl) modifications.

SEQ ID NO:	Sequence (5'-3')	Target
24	<b>AsTsGs C<sup>m</sup>sAsTs TsCs<sup>m</sup>Ts GsCs<sub>m</sub></b>	mouse
	<b>Cs<sup>m</sup> Cs<sup>m</sup>Cs<sup>m</sup>Cs<sup>m</sup>s AsAsGs GsA</b>	C-raf
25	GsC <sup>m</sup> sC <sup>m</sup> s C <sup>m</sup> sAsAs GsC <sup>m</sup> sTs	human
	GsGsC <sup>m</sup> s AsTsC <sup>m</sup> S C <sup>m</sup> sGSTs	ICAM-1
	C <sup>m</sup> SA	

[0276] All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-S-Me- modification. Superscript m on C (Cm) indicates a 5-methyl-C.

Table XI

## Oligonucleotides Containing chimeric

## 2'-O-(2-methoxyethyl) and 2'-O-(methyl) modifications

SEQ ID NO:	Sequence (5'-3')	Target
24	<b>AsTsGs C<sup>m</sup>sAsTs TsCs<sup>m</sup>Ts</b>	mouse
	<b>GsCs<sup>m</sup>Cs<sup>m</sup> Cs<sup>m</sup>Cs<sup>m</sup>Cs<sup>m</sup>s AsAsGs</b>	C-raf
	<b>GsA</b>	

25                   GsC<sup>m</sup>sC<sup>m</sup>s C<sup>m</sup>sAsAs GsC<sup>m</sup>sTs human  
                      GsGsC<sup>m</sup>s ASTsC<sup>m</sup>S C<sup>m</sup>sGSTs ICAM-1  
                      C<sup>m</sup>SA

[0277]           All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-Methyl modification. Superscript m on C (Cm) indicates a 5-methyl-C.

**Table XII**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-ara-(fluoro) modifications**

SEQ ID NO:	Sequence (5'-3')	Target
24	AsTsGs C <sup>m</sup> sAsTs TsCs <sup>m</sup> Ts GsCs <sup>m</sup> Cs <sup>m</sup> Cs <sup>m</sup> C <sup>m</sup> sC <sup>m</sup> s AsAsGs GsA	mouse C-raf
25	GsC <sup>m</sup> sC <sup>m</sup> s C <sup>m</sup> sAsAs GsC <sup>m</sup> sTs GsGsC <sup>m</sup> s ASTsC <sup>m</sup> S C <sup>m</sup> sGSTs C <sup>m</sup> SA --	human ICAM-1

[0278]           All nucleosides in bold are 2'-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-ara-(fluoro) modification. superscript m on C (Cm) indicates a 5-methyl-C.

**Table XIII**  
**Oligonucleotides Containing chimeric**  
**2'-O-(2-methoxyethyl) and 2'-ara-(OH) modifications**

SEQ ID NO:	Sequence (5'-3')	Target
24	AsTsGs C <sup>m</sup> sAsTs TsCs <sup>m</sup> Ts GsCs <sup>m</sup> Cs <sup>m</sup> Cs <sup>m</sup> C <sup>m</sup> sC <sup>m</sup> s AsAsGs GsA	mouse C-raf